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A Fourth Genetic Element to Facilitate Hybrid Cereal Production—A Recessive Tall in Rice[1]

J. N. Rutger[2] and H. L. Carnahan[3]

ABSTRACT

A recessive gene in rice (*Oryza sativa* L.) for elongated uppermost internode (*eui*), which effectively results in a recessive tall plant type, is described. The gene produces a near-doubling in length of the uppermost internode, a 12% increase in panicle length, and little or no effect on other internodes or plant characters. It is proposed that this gene would be a useful fourth element which would complement the other three genetic elements—cytoplasmic male sterility, maintainers, and restorers—generally used in hybrid seed production. The *eui* gene would be incorporated in pollen fertility restoring parents in hybrid seed production in situations where a semidwarf F_1 generation is desired. The tall paternal plant type would be desirable for windblown pollen dispersal onto semidwarf female plants and the resulting hybrid plants would be semidwarf, unlike the usual case of tall hybrids from semidwarf by tall crosses. Additionally, its increased height would permit a co-mingling of the hybrid parent seed stocks to maximize crossing and would facilitate mechanical removal of the paternal parent before mass harvest of the commercial hybrid seed.

Additional index words: Elongated internodes, Male sterility, Fertility restoration, *Oryza sativa* L.

THREE elements, namely cytoplasmic male sterility, a genetic system that maintains the male sterility, and a genetic system that restores male fertility are generally accepted as the necessary elements for producing commercial hybrid seed of crops in which the seed is the end commercial product. Such elements have been used successfully in corn (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench].

A major limitation in the successful use of the above three elements in wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and other naturally self-pollinated cereals is insufficient pollen dispersal to ensure economic production of hybrid seed. In these highly self-pollinated species, increased height of pollinator parents would obviously enhance pollen dispersal by wind and gravity onto short maternal plants.

However, tall mature plant height is usually dominant to short height in cereals. This results in tall hybrid plants in short by tall crosses, which is an undesirable height in situations where short stature plants are known to be more productive. Exceptions to the usual case of dominance for tall height have been reported in rice (Okuno and Kawai, 1978a, 1978b; Mallick et al., 1980), oats (*Avena sativa* L.) (Brown et al., 1980), and wheat (Morris et al., 1972). Okuno and Kawai (1978a, 1978b) observed a 3 short culm: 1 long culm ratio in the F_2 of a cross between LM-1 (long culm mutant 1) and its short culm parent, 'Norin 8.' The recessive tall LM-1 mutant also showed undesired pleiotropic or closely linked effects for late maturity, small panicle number, poor panicle exsertion, long panicle, and long grain. The LM-1 mutant, which was about 23 cm taller than its parent, was classi-

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[2] Research geneticist, AR, SEA, USDA, Davis, CA 95616.

[3] Director of plant breeding, Calif. Co-op. Rice Res. Found., Inc., Biggs, CA 95917.

fied as a "lower-elongation" type, in which the increased height was due to greater elongation of the lower internodes. Mallick et al. (1980) induced two dominant dwarf mutants with reduced height in the semidwarf cultivar 'IR8.' These reduced height mutants were due to a simply inherited, partially dominant gene. The reduced height resulted from a reduction of internode length of the top four internodes. The mutants had undesirable reductions in spikelet length, number of seeds per spike, and 1,000 filled grain weight that were apparently associated with the reduced height locus. Brown et al. (1980) induced a single dominant gene for semidwarfism in oats. The semidwarf was 34% shorter than its parent line, with the height reduction being due to shorter internodes. Morris et al. (1972) found that the wheat cv. 'Tom Thumb' carried a semidominant gene for dwarf stature.

The present report describes an unusual tall plant type in rice and its inheritance as a simple recessive. A method of utilizing this or other recessive talls as a fourth element in the production of hybrid rice seed and similar applications in other cereals are described.

MATERIALS AND METHODS

Two rows containing several plants with very long upper internode (Fig. 1) were discovered by the junior author in the F_3 generation of the cross 'M9'/'Terso,' grown in a breeding nursery in 1976 at the Rice Experiment Station, Biggs, Calif. Progenies of this cross had been selected in the F_2 generation for the semidwarf habit of the M9 parent, which derived its semidwarfism from 'IR8' (Carnahan et al., 1978). Believing that this plant type might have value in hybrid seed production, one plant with elongated internode, designated 76:4512 (P_1), was selected for genetic studies. Since line 76:4512 was homozygous for the recessive marker character glabrous leaf, it was used as the maternal parent in a cross with ED7 (P_2), a semidwarf pubescent leaf line. ED7 is a spontaneous mutant for early maturity, selected from the cv. 'Calrose 76,' which is in turn an induced short stature mutant from the tall cv. 'Calrose' (Rutger et al., 1977). Three F_1 plants were grown in the greenhouse. As expected the plants were pubescent, showing that they were hybrids. No measurements were made on the F_1 plants, which were phenotypically similar in height to their male semidwarf parent.

The F_2 generation was grown in 1979 at the Rice Research Facility at Davis. Each plant was visually scored for height and leaf pubescence. Plant height, panicle length, and internode length were measured on a random subsample of 154 F_2 plants (116 normal semidwarfs: 38 elongated uppermost internodes), plus both parents (20 P_1 and 27 P_2 plants). The F_3 progeny were grown in Hawaii during the winter of 1979-1980 to determine the genotype of each F_2 plant. Thirteen progeny of each F_2 plant were transplanted in the Hawaiian nursery, but a storm reduced the progeny number to less than eight plants in 48 of the 154 lines. The eight-plant lower limit was chosen because a progeny size of at least eight plants is required in order to have a 90% chance of detecting at least one recessive plant in a population segregating 3 dominant:1 recessive. The remaining 106 F_2 plants were found to segregate 34 homozygous semidwarf normal:50 heterozygous:22 homozygous elongated uppermost internode ($x^2 = 3.05$, NS with 2df). The averages of the two types of homozygous F_2's provided a near-isogenic comparison for the effects of the elongated uppermost internode gene, and the average for the heterozygous F_2 plants provided a measure of degree of dominance. A one-way analysis of variance was conducted on the F_2 data, with each of the three genotypes as treatments, and unequal numbers of replications (plants) per treatment.

RESULTS AND DISCUSSION

Inheritance and Characterization

The F_2 segregaton fit a 3 normal:1 elongated uppermost internode ratio (Table 1), indicating that tallness was controlled by a single recessive gene. We propose the gene symbol *eui* for this elongated uppermost internode character. Segregation for pubescence fit the expected 3 pubescent:1 glabrous leaf type. The chi-square test for linkage showed that internode type and pubescence were independent. The progeny tests of 22 *eui* F_2 plants produced only *eui* F_3 plants, and the progeny tests of 84 normal semidwarf F_2 plants produced the expected short:tall segregation in 50 families and short plants only in the other 34 families.

Table 1. Classification of F_2 plants of the cross 76:4512/ED7 for elongated uppermost internode and leaf pubescence.

Leaf type	Uppermost internode: Normal semi-dwarf type	Uppermost internode: Elongated type	Total
Pubescent	343	113	456
Glabrous	121	39	160
Total	464	152	616

χ^2 for 3:1 inheritance of pubescent vs. glabrous = 0.31, 0.50 < P < 0.75
χ^2 for 3:1 inheritance of normal vs. elongated = 0.04, 0.75 < P < 0.90
χ^2 for linkage, 1 df = 0.01, 0.90 < P < 0.95

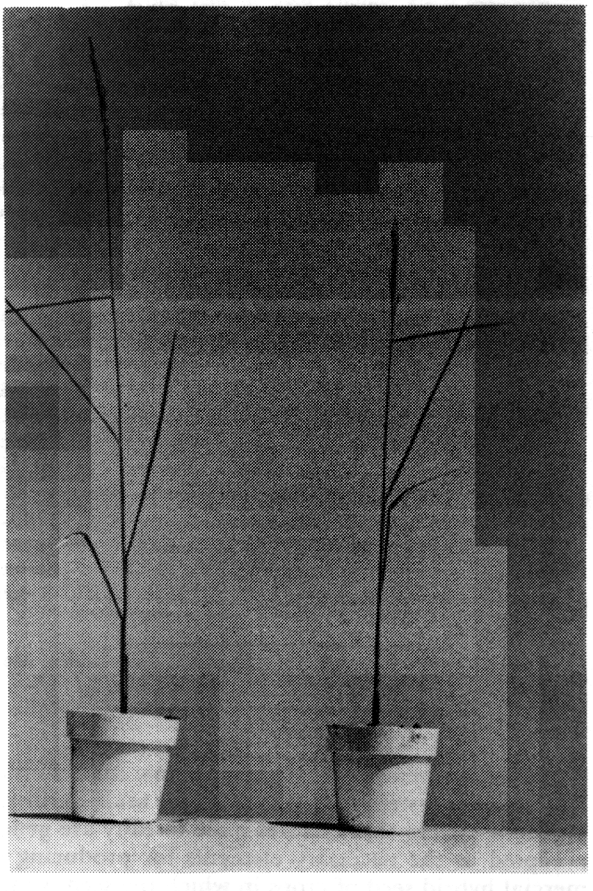

Fig. 1. Single culm of a rice plant with elongated uppermost internode (left) compared with a culm of a normal semidwarf plant (right).

Internode measurements showed that the tall plant height of P_1 was due to a near-doubling of the length of the uppermost internode, when compared to P_2, the semidwarf parent (Fig. 2). Panicle exsertion above the flag leaf was greatly increased by the elongated internode. Lengths of the other internodes were similar to those of P_2.

The analysis of variance on the three F_2 genotypes showed highly significant differences for total plant height, first internode length, and panicle length, but no significant differences for second, third and fourth internode length (Table 2). All the significant differences were due to larger values for the tall genotype, *eui/eui* (Fig. 2). Differences between the two short genotypes, *Eui/eui* and *Eui/eui*, were not significant, demonstrating that *eui* is completely recessive to *Eui*.

The comparison of the *eui/eui* F_2 homozygotes vs. the *Eui/Eui* F_2 homozygotes provided an excellent measure of the effects of this gene locus. Thus the *eui/eui* genotypes not only were taller and had much longer uppermost internodes than the *Eui/Eui* genotypes, but they also had about 12% longer panicles (20.6 vs. 18.4 cm, respectively). No other effects of the *eui* gene were evident.

Table 2. Mean squares for components of height from the analysis of variance on the three F_2 height genotypes from the cross 76:4512/ED7.

Source	df	Plant height	Panicle length	Internode length 1st	2nd	3rd	4th
Genotypes	2	5,831.3**	40.4**	3,960.5**	10.6	7.3	1.8
Error	103	25.2	2.1	8.1	3.5	2.8	4.0
L.S.D.$_{0.01}$ (cm) for comparing *eui/eui* and *Eui/Eui*		3.5	1.0	2.0	–	–	–

** $P < 0.01$.

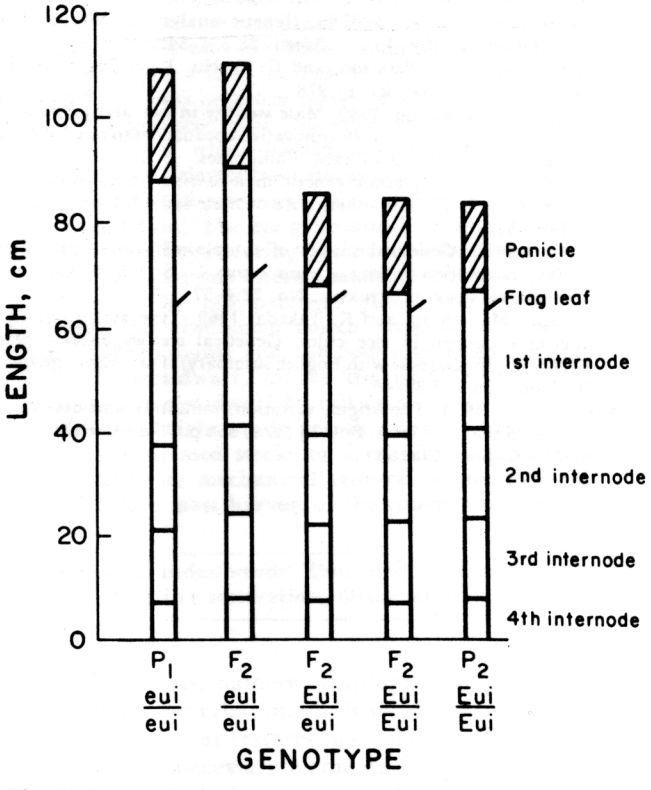

Fig. 2. Internode lengths, panicle length, and position of flag leaf of parents and F_2 genotypes from the cross between an elongated uppermost internode parent and a normal semidwarf parent.

Since it was not observed in either parent, the *eui* character may have arisen either from complementary gene action, or as a sponstaneous mutation. The pattern of near-doubling of length of the uppermost internode of the *eui/eui* genotype is completely distinctive from the recessively-inherited elongated lower internode pattern of the induced long-culm mutant LM-1 reported by Okuno and Kawai (1978b). The internode pattern of the *eui/eui* genotype is also distinctive from the reduced top four internode pattern of the dominant dwarfs reported by Mallick et al. (1980), as well as being distinctive from the internode patterns reported by Takahashi and Takeda (1969). Additionally, the present *eui* gene had little or no effect on other characters, in contrast to the several pleiotropic effects observed by Okuno and Kawai (1978b) in their genetic analyses.

The present study does not differentiate whether the elongated uppermost internode characteristic results from a gene nonallelic to the semidwarfism gene (sd_1) present in ED7 and probably in 76:4512, or from a different allele at the sd_1 locus. The distinction between these possibilities can be made by crossing *eui/eui* plants with normal tall (Sd_1Sd_1) plants. The appearance of *eui* plants taller than the tall parent in the F_2 of such a cross would indicate a gene nonallelic to Sd_1. The flag leaf of such *eui* segregates would be approximately at the same level as the flag leaf on the normal tall. Conversely, the appearance of *eui* plants of total height similar to the tall plant would be indicative of another allele at the sd_1 locus. In this latter case, the flag leaf height of the *eui* segregates would be expected to be similar to those of the normal sd_1 semidwarf.

Utilization in Hybrid Seed Production

The recessive tall characteristics of this elongated internode genotype may be useful as a fourth element in hybrid rice seed production. The first three elements considered necessary for hybrid rice production, cytoplasmic male sterility, maintainers, and restorers, have been known for some time. Cytoplasmic male sterility in rice was first reported by Katsuo and Mizushima (1958). Subsequently at least eight additional combinations of cytoplasmic male sterility in rice, including two combinations in California (Erickson, 1969), have been reported (Rutger and Shinjo, 1980). Shinjo (1969, 1975) was the first to report the genetics of fertility restoration. Commercial production of hybrid rice seed in People's Republic of China was reported by Lin and Yuan (1980). As with other crops, a fertility restorer line is used as pollinator parent of the final cross.

Athwal and Virmani (1972) emphasized the problems of obtaining commerical quantities of hybrid rice seed as follows: "Perhpas the most important problem is that of modifying the floral structure of male-sterile rice to increase its outcrossing potential." They also suggested "If a dominant gene for semidwarfing is discovered, it will

facilitate development of hybrids with high yield potential."

We propose that the recessive gene for elongated internode could be incorporated into the restorer parent of hybrid rice. Then this parent would be interplanted with the male sterile seed parent in commercial hybrid seed production fields. Tall male parents have an obvious advantage in pollen dispersal in seed production fields, but the tall F_1's that would result from normal dominant tall pollinators are unwanted in the hybrid in vast areas where semidwarf cultivars are known to be more productive than tall cultivars. In contrast, use of the present recessive *eui* gene in pollinator parents results in the desired short F_1 hybrids for the commercial crop. Furthermore, since the pollinator is about 25 cm taller than the female parents, it should be possible to eliminate most of the pollinators by clipping off their panicles or by rope-wick application of herbicide soon after pollination. The *eui*/*eui* pollinators must be removed soon after pollination, because they eventually bend down into the canopy as grain filling proceeds. After the pollinators are removed, the entire field can be harvested for hybrid seed production. Undoubtedly, some tall plants would be missed in the removal process, but presumably a small percentage of tall plants would be tolerable in the F_1 field if sufficient heterosis occurs to make hybrids feasible.

Heterosis is widespread in the plant kingdom, including rice (Athwal and Virmani, 1972; Carnahan et al., 1972; Davis and Rutger, 1976; Rutger and Shinjo, 1980). However, these authors correctly emphasized that their studies and those reviewed for rice were not carried out under commercial planting conditions nor with presently available, high yielding semidwarf materials and were therefore of limited value. On the other hand, the reported 5 million ha of hybrid rice in China strongly suggest the existence of worthwhile heterosis (Lin and Yuan, 1980).

The principle of recessive talls may also be applied in other cereals. Vavilov (1951) proposed the law of homologous series and the concept of parallel variation. He listed 28 contrasting characters that were present in both wheat and rye, cereals belonging to different genera. Vavilov (1951) cited many additional examples of parallel variation widespread in the plant kingdom. Similarly, parallel cytoplasmic male sterility, maintainer systems, and fertility restorer systems have been found in corn, sorghum, wheat, barley (*Hordeum vulgare* L.), and other plants.

Thus, we propose that recessively inherited tall plants can be found in other cereal crops such as wheat and barley, and that such tall plants can be employed in the successful production of hybrid seed of those crops as well as of rice.

REFERENCES

Athwal, D. S., and S. S. Virmani. 1972. Cytoplasmic male sterility and hybrid breeding in rice. p. 615-620. *In* Rice Breeding, Int. Rice. Res. Inst., Los Baños, Philippines.

Brown, P. D., R. I. H. McKenzie, and K. Mikaelsen. 1980. Agronomic, genetic, and cytologic evaluation of a vigorous new semidwarf oat. Crop Sci. 20:303-306.

Carnahan, H. L., J. R. Erickson, S. T. Tseng, and J. N. Rutger. 1972. Outlook for hybrid rice in the USA. p. 603-607. *In* Rice Breeding, Int. Rice Res. Inst., Los Banos, Philippines.

– – – –, C. W. Johnson, S. T. Tseng, and J. J. Mastenbroek. 1978. Registration of 'M9' rice. Crop Sci. 18:357-358.

Davis, M. D., and J. N. Rutger. 1976. Yield of F_1, F_2 and F_3 hybrids of rice (*Oryza sativa* L.). Euphytica 25:587-595.

Erickson, J. R. 1969. Cytoplasmic male sterility in rice (*Oryza sativa* L.). Am. Soc. Agron. Abstr. p. 6.

Katsuo, K., and U. Mizushima. 1958. Studies on the cytoplasmic difference among rice varieties, *Oryza sativa* L. I. On the fertility of hybrids obtained reciprocally between cultivated and wild varieties. (In Japanese). Jpn. J. Breed. 8:1-5.

Lin, Shih-Cheng, and Loung-Ping Yuan. 1980. Hybrid rice breeding in China. p. 35-51. *In* Innovative approaches to rice breeding. Int. Rice Res. Inst., Los Baños, Philippines.

Mallick, E. H., N. Gosh Hijra, and P. Bairagi. 1980. Genetic analysis of the mutant character (Induction of dominant dwarf mutation in rice). Il Riso 28(1):3-7.

Morris, R., J. W. Schmidt, and V. A. Johnson. 1972. Chromosomal location of a dwarfing gene in 'Tom Thumb' wheat derivative by monosomic analysis. Crop Sci. 12:247-249.

Okuno, K., and T. Kawai. 1978a. Variations of internode length and other characters in induced long-culm mutants of rice. Jpn. J. Breed. 28:243-250.

– – – –, and – – – –. 1978b. Genetic analysis of induced long-culm mutants in rice. Jpn. J. Breed. 28:336-342.

Rutger, J. N., M. L. Peterson, and C. H. Hu. 1977. Registration of Calrose 76 rice. Crop Sci. 17:978.

– – – –, and C. Shinjo. 1980. Male sterility in rice and its potential use in breeding. p. 53-66. *In* Innovative approaches to rice breeding. Int. Rice Res. Inst., Los Baños, Philippines.

Shinjo, C. 1969. Cytoplasmic-genetic male sterility in cultivated rice, *Oryza sativa* L. II. The inheritance of male sterility. Jpn. J. Genet. 44:149-156.

– – – –. 1975. Genetical studies of cytoplasmic male sterility and fertility restoration in rice, *Oryza sativa* L. Sci. Bull. College of Agriculture, Univ. of Ryukyus, No. 22:1-57.

Takahashi, Man-emon, and K. Takeda. 1969. Type and grouping of internode pattern in rice culm. Genetical studies on rice plant, XXXVII. (In Japanese with English summary). Fac. Agric. memoirs, Hokkaido Univ. 7:32-43.

Vavilov, N. I. 1951. The origin, variation, immunity and breeding of cultivated plants. Chron. Bot. 13:(1/6) 364 p. (Translated from Russian by K. Starr Chester).